DNA 3130 SPECTRAL CALIBRATION USING DS-36 (GLOBALFILER)

A. SCOPE

A spectral calibration creates a matrix that corrects for the overlapping fluorescence emission spectra of the dyes. Although each of these dyes emits its maximum fluorescence at a different wavelength, there is some overlap in the emission spectra between the dyes. The goal of multicomponent analysis is to effectively correct for spectral overlap. Performing a spectral calibration is similar to performing a sample run, except that calibration standards are run in place of samples and a spectral calibration module is used in place of a run module.

B. QUALITY CONTROL

B.1 See DOC ID 1835 to determine reagent expiration dates.

B.2 Do not clean any components or accessories of the 3130 with bleach or ethanol. Clean with deionized water.

B.3 Hi-Di Formamide: To prevent repeated thaw and re-freezing of formamide, aliquot formamide into approximately 500 and 1000 µL volumes after initially thawing the 25 mL bottle. Appropriately discard any unused aliquot of thawed formamide.

C. SAFETY

C.1 Hi-Di Formamide: exposure causes eye, skin, and respiratory tract irritation. It is a possible developmental and birth defect hazard.

C.2 All appropriate SDS sheets must be read prior to performing this procedure.

C.3 Protective gloves, a lab coat eye protection (e.g. safety glasses or a face shield) must be worn at all times when performing this procedure.

C.4 Distinguish all waste as general, biohazard, or sharps and discard appropriately.

D. REAGENTS, STANDARDS, AND CONTROLS

D.1 3130 Performance Optimized Polymer (POP-4 polymer)

D.2 DS-36 Matrix Standard (Dye Set J6) for GlobalFiler which includes the following dyes: 6FAM, VIC, NED, SID, TAZ and LIZ

D.3 AB Prism 10X Genetic Analyzer Buffer w/ EDTA to make a 1X working buffer:

Add 25 mL of Buffer 10X to 225 mL of deionized water to make 250 mL of working buffer, or make up a 1000 mL of the working buffer by adding 100 mL of Buffer 10X (4 bottles) to 900 mL deionized water.

D.4 Hi-Di Formamide

D.5 Deionized water

E. EQUIPMENT & SUPPLIES

E.1 Equipment

E.1.1 AB 3130 Genetic Analyzer (instrument, computer and appropriate software)
E.1.2 AB 36 cm capillary array
E.1.3 AB Prism Genetic Analyzer sample septa and plates
E.1.4 Thermal cycler
E.1.5 Pipettes
E.1.6 Vortexer
E.1.7 Frozen plate block
E.1.8 96-well plate retainer and base
E.1.9 96-well plate centrifuge
E.1.10 Microcentrifuge

E.2 Supplies

E.2.1 3130 Genetic Analyzer buffer vials/reservoirs/reservoir septa
E.2.2 Pipette tips
E.2.3 Microcentrifuge tubes
E.2.4 Scalpel

F. PROCEDURES

NOTE: A spectral calibration using DS-36 (GlobalFiler), DS-33 (YFiler, DOC ID 1768) and the PowerPlex matrix standards (DOC ID 1769) may be prepared and run on the same plate; however, the PowerPlex matrix standards must not be added to the plate until the denaturation and snap cooling of the DS36 and DS33 matrix standards are complete. In addition, when running all three spectrals, the oven temperature must be set to 60ºC so that the oven can be preheated for at least 15 minutes prior to the first injection (necessary for the PowerPlex matrix standards).

F.1 Thoroughly mix the contents of the DS-36 tube and then spin briefly in a microcentrifuge.

F.2 Combine 3 µl of DS-36 spectral standard (includes 6-FAM, VIC, NED, SID, TAZ and LIZ dyes) with 297 µl of formamide. Vortex thoroughly and then spin briefly in a microcentrifuge.

F.3 Dispense 10 µl of the spectral standard/formamide mixture into 4 wells of a 96 well plate (one per capillary).

F.4 Cover the plate and denature for 3 minutes at 95 °C, immediately place in the frozen plate block for 3 minutes.

F.5 Centrifuge the plate, use a retainer clip to secure it onto the plate base, and place this on a 3130 instrument for a run.

F.6 In **Plate Manager** select, **new**, and name the run appropriately, e.g. Spectral-mmddyy.

F.7 Under application select **Spectral Calibration.**

F.8 Select the plate default value of **96 well**. Add operator initials, select **OK.**

F.9 Fill in the respective plate locations of the spectral standard e.g. A01-D01 with the sample name DS36.

F.10 Select instrument protocol as **Spectral-DS-36**.

F.11 Go to **Run Scheduler** select **find all,** highlight the plate document created in plate manager and link the plate document to the yellow plate by mouse clicking on the plate diagram. Select the green arrow to run.

G. INTERPRETATION GUIDELINES

G.1 Upon completion review the pass or fail status of each capillary in the **Instrument status/ Events Messages**. In a good quality calibration each capillary should have a Quality-value (Q-value) above 0.95 and a condition number up to 8.0. A Q-value of 1 indicates an ideal matrix providing no detectable pull-up/pull-down and a condition number less than 8.0 shows that the amount of overlap between the dye peaks in the dye set is acceptable.

G.2 If the entire spectral failed re-make the DS-36 spectral standard/formamide mixture and repeat the spectral calibration procedure.

G.3 Go to **Spectral Viewer**, ensure the Dye set is J6, and select a well position that had spectral standards in it from the plate layout diagram. A green box in the sample position indicates that the capillary passed and a gray-brown box indicates that it failed.

G.4 For the selected capillary, verify that the order of the peaks in the spectral profile (pixel vs. signal intensity) from left to right are blue, green, yellow, red, purple and orange.

G.5 Verify that the peaks in the spectral profile do not contain gross overlaps, dips, or other irregularities.

G.6 If acceptable click **rename** and rename the spectral run with the date of the run.

G.7 Click **OK**.

H. REFERENCES

H.1 Multi-Capillary DS-36 Matrix Standard (Dye Set J6) Publication Number 4426042, Applied Biosystems by Life Technologies, July 2012.